REMARKS

Status of the Claims.

Claims 1-31 are pending with entry of this amendment, claims 32-61 being cancelled and no claims being added herein. Claims 1, 3, 15, and 28 are amended herein. These amendments introduce no new matter. Support is replete throughout the specification and in the claims as originally filed.

Election/Restriction.

Pursuant to a restriction requirement made final, Applicants cancel claims 32-61 with entry of this amendment. Please note, however, that Applicants reserve the right to file subsequent applications claiming the canceled subject matter and the claim cancellations should not be construed as abandonment or agreement with the Examiner's position in the Office Action.

Claims 4, 26-27, and 32-61 were withdrawn from further consideration pursuant to 37 C.FR. §1.142(b) as being drawn to a non-elected invention, there allegedly being no allowable generic or linking claim. Applicants note that claim 4 as amended herein is allowable and generic to claims 4, 26 and 27. Accordingly Applicants request that these claims be reinstated.

Information Disclosure Statement.

Applicants note with appreciation the Examiner's thorough consideration of the references cited in the Information Disclosure Statements (Forms 1449) submitted on November 11, 2003 and August 26, 2002.

35 U.S.C. §112, Second Paragraph.

Claims 1, 3, and 2, 5-25, 28-31 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite because claims 1 and 3 recite "on a cell comprising the blood brain barrier." According to the Examiner one of skill cannot determine what is meant by this limitation because the blood brain barrier is comprised of cells and cannot envision the metes and bound of a claim drawn to a single cell that comprises the entire blood brain barrier. In addition the Examiner alleged that there is no antecedent basis for the limitation of "said cDNA" in this claim.

While Applicants disagree with the Examiner's interpretation of the term comprise, to expedite prosecution Applicants have amended claims 1 and 3 to recite ". . . a cell composing the blood brain barrier . . . " thereby obviating this rejection. In this regard, it is noted, for example that Dictionary.com states:

> ***Usage Note:*** The traditional rule states that the whole *comprises* the parts and the parts *compose* the whole. In strict usage: *The Union comprises 50 states. Fifty states compose (or constitute or make up) the Union.* Even though careful writers often maintain this distinction, ***comprise* is increasingly used in place of *compose*, especially in the passive: *The Union is comprised of 50 states***. Our surveys show that opposition to this usage is abating. In the 1960s, 53 percent of the Usage Panel found this usage unacceptable; **in 1996, only 35 percent objected**.

Claim 1 is also amended herein to eliminiate reference to "said DNA" thereby obviating the rejection under 35 U.S.C. §112, second paragraph, on these grounds.

Claims 2 and 15 were rejected under 35 U.S.C. §112, second paragraph, because there was allegedly insufficient antecedent basis for the limitation "said nucleic acid". Claims 2 and 15 are amended herein to recite "said first nucleic acid" thereby obviating this rejection.

Claim 28 was rejected under 35 U.S.C. §112, second paragraph, because there was allegedly insufficient antecedent basis for the limitation "said contacting". Claim 28 is amended herein to recite "said administering" thereby obviating this rejection.

35 U.S.C. §102.

A) Penichet *et al.*

Claims 1-3, 6-16, 18-24, and 28-30 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Penichet *et al.* (1999) *J. Immunol.,* 163: 4421-4426. Applicants traverse.

Claim 1, as amended herein recites:

> 1: A method of imaging *in vivo* expression of a gene in a brain cell of a vertebrate, said method comprising:
> i) administering to said vertebrate an imaging reagent comprising a detectable label attached to a first nucleic acid that specifically hybridizes to a second nucleic acid transcribed from said gene, where said first nucleic acid

> is linked to a targeting ligand that binds a receptor on a cell composing the blood brain barrier of said vertebrate, **whereby said composition crosses said blood brain barrier and enters a brain cell and said first nucleic acid specifically hybridizes to said second nucleic acid**; and
>
> ii) detecting the presence or quantity of a signal produced by said detectable label in said brain cell where the presence or quantity of said label indicates the presence or quantity of said nucleic acid transcribed from said gene. [emphasis added]

Penichet et *al.* discloses the administration of a compositon comprising [^{125}I]biotin-PNA bound to anti-TfR *IgG3-C_H3-Av* to a rat:

> Experiments were then performed to determine whether the anti-TfR IgG3-C_H3-Av fusion protein can be used to deliver a biotinylated 18-mer **antisense specific for the *rev* gene of HIV-1** (biotin-PNA), a molecule with therapeutic potential against HIV, to the brain. [^{125}I]Biotin-PNA was injected i.v. into rats with or without anti-TfR IgG3-C_H3-Av, and the brain uptake was analyzed as described above (Table III). The brain uptake of unconjugated [^{125}I]biotin-PNA was negligible, with a PS product of 0.12 ± 0.01 $\mu l \cdot min^{-1} \cdot g^{-1}$ and a brain uptake of 0.0083 ± 0.0009% ID/g. In contrast, the brain uptake of [^{125}I]biotin-PNA bound to anti-TfR IgG3-C_H3-Av was 0.12 ± 0.01% ID/g at 60 min after an i.v. injection, and its BBB PS product was 0.67 ± 0.09 $\mu l \cdot min^{-1} \cdot g^{-1}$. The PS product for the [^{125}I]biotin-PNA was increased 5.6-fold, and brain uptake was increased 14.5-fold when the [^{125}I]biotin-PNA was bound to anti-TfR IgG3-C_H3-Av. Thus, this novel Ab-Av fusion protein can deliver the biotinylated antisense drug anti-HIV PNA across the BBB, suggesting that brain delivery of anti-HIV PNA with the anti-TfR IgG3-C_H3-Av may provide an effective treatment for cerebral AIDS.

The PNA comprising the Penichet construct was an "1**8 mer antisense specific for the rev gene of HIV-1**." As rats in general cannot support HIV and the rats in Penichet *et al.* were not infected for HIV, **there was no target present in the rat brains for the PNA component** of the Penichet *et al.* construct.

Consequently, Penichet *et al.* fails to disclose a method in which " . . . said composition crosses said blood brain barrier and **enters a brain cell** and **said first nucleic acid specifically hybridizes to said second nucleic acid** . . . " as recited in claim 1. Penichet *et al.* thus fails to provide all the elements of the presently claimed invention and consequently the rejection under 35 U.S.C. §102(b) on these grounds should be withdrawn.

B) Pardridge *et al.* (1995).

Claims 1-3, 6-16, 18-24, and 28-30 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Pardridge *et al.* (1995) *Proc. Natl. Acad. Sci., USA,* 92: 5592-5596. Applicants traverse.

Pardridge *et al.* also fails to disclose a method that involves:

> i) administering to said vertebrate an imaging reagent comprising a detectable label attached to a first nucleic acid that specifically hybridizes to a second nucleic acid transcribed from said gene, where said first nucleic acid is linked to a targeting ligand that binds a receptor on a cell composing the blood brain barrier of said vertebrate, **whereby said composition crosses said blood brain barrier and enters a brain cell and said first nucleic acid specifically hybridizes to said second nucleic acid**;

as recited in claim 1.

Like Penichet *et al.*, Pardridge discloses an 18 mer PNA that binds to an **HIV *rev* mRNA** attached to an antibody that binds to a transferring receptor. The antibody-PNA construct is administered to a rat. Again, **there is no HIV and consequently no target for the PNA in the rat brain.** Pardridge *et al.* thus fails to disclose a method where ". . . said composition crosses said blood brain barrier and enters a brain cell and said first nucleic acid specifically hybridizes to said second nucleic acid. . . " as recited in the presently pending claims.

Moreover, the ability of the PNA to hybridize to target mRNA following biotinylation and binding to OX26-SA was demonstrated by RNase A/T1 protection assay (see page 5595, col. 2). This assay is performed *in vitro:*

> For the RNAse protection assay, 0.5 pmol of either biotinylated or nonbiotinylated PNA as incubated with or without 10 pmol of OX26-SA for 5 min on ice in 19 μl of reaction buffer (0.3 M NaCl/10 mM Tris, pH 7.4/4 mM EDTA/0.02%tRNA); 10^5 cpm of ^{32}P-labeled sense of antisense rev RNA (4.2 or 5.9 fmol, respectively) was added in 1 μl of reaction buffer and annealed for 30 min at 42 °C. Twenty units of RNase T1 and 2.5 μg of RNase A were added to the samples in 10 μl of reaction buffer containing 1.5 μg of bovine serum albumin and digestion of unprotected RNA was carried out for 30 min at 37°C. (page 5593, col. 2)

Consequently Pardridge *et al.* fails to disclose a method where the construct hybridizes to its target nucleic acid in a brain cell. Pardridge *et al.* thus fails to disclose all of the elements of the presently pending claims and the rejection under 35 U.S.C. §102(b) should be withdrawn.

35 U.S.C. §103(a).

Claims 1-3, 5-25, and 28-31 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Penichet *et al.* (1999) *J. Immunol.,* 163: 4421-4426 and Pardridge *et al.* (1995) *Proc. Natl. Acad. Sci., USA,* 92: 5592-5596 as applied to claims 1-3, 6-16, 18-24, and 28-30 (under 35 U.S.C. §102(b)) and further in view of Hnatowich (1999) *J. Nucl. Med.,* 40: 693-703, Kurihara and Pardridge (1999) *Cancer Res.,* 54: 6159-6163, and Tavitian (1998) *Nat. Med.,* 4: 467-471. According to the Examiner Penichet *et al.* and Pardridge *et al.* (1995) fail to teach the method where the imaging reagent comprises a targeting ligand that is an antibody that specifically binds to an insulin receptor, where the nucleic acid is labeled with a radio labeled amino acid that is 111-indium, or wherein the vertebrate is a human.

Kurihara *et al.* is cited as allegedly teaching the directed targeting of an EGF peptide radiopharmaceutical to image brain tumor where delivery is enabled to undergo transport through the blood brain barrier (BBB) because of conjugation of a monoclonal antibody that transcytoses through the BBB and the EGF peptide is labeled with ^{111}In. Hnatowich *et al.* alleged teaching antisense as an imaging tool using radiolabeled DNA. Tavitan *et al.* allegedly teach that antisense oligonucleotides are promising new pharmaceuticals that must be modified to avoid rapid degradation and non-specific binding and to allow membrane passage. Applicants traverse.

The Examiner is respectfully reminded that *prima facie* case of obviousness requires that the combination of the cited art, taken with general knowledge in the field, must provide all of the elements of the claimed invention. When a rejection depends on a combination of prior art references, there must be some **teaching, suggestion, or motivation to combine** the references. *In re Geiger*, 815 2 USPQ2d 1276, 1278 (Fed. Cir. 1987). Moreover, to support an obviousness rejection, the cited references must additionally **provide a reasonable expectation of success**. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991), *citing In re Dow Chemical Co.,* 5 USPQ2d 1529, 1531 (Fed. Cir. 1988).

In the instant case, the combination of the cited art **fails** to provide all the element of the claimed invention and also **fails** to provide a reasonable expectation of success.

The presently pending claims are directed to methods of imaging **in vivo expression** of **a gene in a brain cell** of a vertebrate.

In order to image gene expression in vivo, the construct used in the presently claimed methods must cross two barriers in series -- The blood brain barrier (BBB) and the membrane of a brain cell. In addition, the construct must cross these barriers in sufficient quantity that in vivo imaging is possible.

Applicants explain below that the methods disclosed in the cited art:

1) Do not show constructs crossing both the **blood brain barrier** and the **cell membrane**;

2) Do not show **hybridization of such constructs to gene transcripts** (mRNA) in vivo; and

3) Do not establish a **reasonable expectation of success**.

Penichet *et al.*, for example simply discloses the administration of a composition comprising [^{125}I]biotin-PNA bound to anti-TfR *IgG3-C_H3-Av* to a rat. The PNA component is a PNA specific for the *rev* gene of HIV-1.

There is no HIV-1 *rev* target in the mouse brain. Consequently, **the construct disclosed by Penichet *et al.* has no target with which to hybridize**. In view of this, all of the elements of the presently claimed invention are not provided by this reference.

Moreover, while Penichet *et al.* alleges that the construct crosses the blood brain barrier (BBB), this reference does not establish that the construct crosses a cell membrane or that the construct appears in the cytosol of a brain cell in sufficient quantity to permit detection of gene expression.

Indeed, Penichet *et al.* **do not** even attempt detection of the PNA construct inside a brain cell. To the contrary, Penichet *et al.* state:

> The plasma and **brain samples were solubilized** with Soluene-350 (Packard Instrument, Saehan, Korea) and neutralized with glacial acetic acid before liquid scintillation counting. The other peripheral tissues, such as liver, kidney, lung, and heart, were also removed and weighted and their radioactivities were counted. [emphasis added] (page 4423, col. 1)

Since brain tissues were removed and solubilized before scintillation counting, indicates that any extracellular construct would be included in this measurement. Consequently Penichet *et al.* fails to establish that the construct crosses both the BBB and the cell membrane.

Moreover, by teaching extraction and solubilization of brain tissue to detect the labeled construct, Pinichet *et al.* effectively teaches away from a method of detecting *in vivo* gene expression as recited in the presently pending claim.

In addition, Pinichet *et al.* expressly state:

> Nevertheless, the brain uptake of biotin-PNA bound to anti-TfR IgG3-C_H3-Av **was half that** of biotin bound to anti-TfR IgG3-C_H3-Av. The PS product (0.67 μl/min/g brain) of anti-TfR IgG3-C_H3-Av/biotin-PNA descreased to 30% of the PS product (2.25 μl/min/g brain) of anti-TfR IgG3-C*H*3-Av/biotin. **The decreased brain uptake may reflect the poor intrinsic intracellular permeability of the PNA moiety in the complex.** [emphasis added] (page 4426, col. 1)

effectively teaching that uptake of the PNA construct (even across just the BBB) is low and possibly a concern. In view of the unpredictability of the art (prior to the examples provided in the present specification), the failure to establish passage of the PNA construct across the membrane of a brain cell, the failure to show hybridization of the PNA to a gene transcript in a brain cell, Penichet6 *et al.* both fails to provide the presently claimed invention and fails to offer a reasonable expectation of success (*i.e.*, that a construct such as that recited in the pending claims could cross both the BBB and a brain cell membrane in sufficient quantity to hybridize to a target nucleic acid and produce a detectable signal).

These defects are not remedied by the remaining references. Pardridge *et al.* (1995) for examples offers essentially the same teaching as Pinichet *et al.* Pardridge *et al.* discloses administration of essentially the same construct (an anti *HIV-1 rev* PNA coupled to the OX26-streptavidin conjugate) to a rat. This reference simply demonstrates that the construct crosses the blood brain barrier. No evidence is presented to show that the construct also crosses a cell membrane or that is does so in sufficient concentration to permit detection of gene expression. Indeed, this reference merely states:

> Our results are consistent with eth following conclusions. (*i*) Free PNAs have very low rates of clearance by brain and other organs (except kidney) and are largely excreted into the urine within 60 min after an intravenous injection. (*ii*) Binding of bio-PNA to the OPX26-SA vector redirects PNA delivery from kidney to rorgans with abundant transferring receptors, such as liver of the BBB. (*iii*) The bio-PNA bound to OX26-SA is metabolically stable, as

> shown by measurement of serum TCA-precipitable radioactivity (Fig. 2 Right) and serum HPLC analysis (FIg. 1B). (*iv*) Conjugation of bio-PNA to OX26-SA does not interfere with the PNA bybridization to target mRNA (Fig. 4).

No mention is made regarding uptake into brain cells of the construct. No mention is made of hybridization to a gene transcript **within brain cells**. Indeed, the only measure of the ability of PNA to hybridize to a target was performed *in vitro* using an RNase protection assay.

The combination of Pinichet *et al.* and Pardridge *et al.* thus fails to show passage of the PNA construct across the membrane of a brain cell, fails to show hybridization of the PNA to a gene transcript in a brain cell, and fails to establish that sufficient construct can be delivered to a brain cell to permit detection of gene expression. The combination of these references thus both fails to provide the presently claimed invention and fails to offer a reasonable expectation of success (*i.e.*, that a construct such as that recited in the pending claims could cross both the BBB and a brain cell membrane in sufficient quantity to hybridize to a target nucleic acid and produce a detectable signal).

The remaining cited references, Kurihara and Pardridge, Hnatowich, and Tavitan *et al.* fail to remedy these defects. Thus Kurihara and Pardridge, for example, disclose the use of an anti-TfR-EGF construct where the EGF is radiolabeled with ^{111}In. Both components of the construct are proteins. **The construct contains no nucleic acid.** In addition, the EGF receptor is found on the **surface** of a cell. Binding of EGF to is cognate receptor does not require the construct to cross the cell membrane.

Consequently Kurihara and Pardridge offers no teaching regarding the ability of a nucleic acid containing construct to cross both the BBB and a cell membrane, to hybridize to a target inside a brain cell, or to be present inside the cell in sufficient quantity to permit detection of gene expression.

Hnatowich discusses the use of labeled nucleic acids for imaging applications. This reference, however, **does not** disclose the use of labeled nucleic acids attached to a targeting ligand as recited in the presently pending claims. Moreover, Hnatowich expressly teaches that *in vivo* imaging with labeled nucleic acids is problematic (even where passage through the BBB is not required). Thus, Hnatowich expressly states:

To achieve therapy or **imaging**, antisense DNAs **must cross the cell membrane and enter the cytoplasm without encapsulation and permanent entrapment in endosomal or lysosomal vesicles**. Generally, only **a small percentage** of DNAs incubated with cells are incorporated under the most favorable circumstances (36,37).

* * *

The **ineffeicient intracellular localization** of antisense phosphorothioate DNAs explaines, in part, the large dosages (e.g. 0.05 mg/kg/h over 10 D) now being administerd to patients in connection with antisense chemotherapy (47).

* * *

For imaging the problem of ineffecient cellular transport may not be so easily resolved , since simply increasing the dosage of radiolabeled DNAs could decrease target/nontarget radioactivity ratios should the excess labeled DNAs accumulate in normal tissues or show delayed clearance from target tissues. [emphasis added] (page 696 col. 2, paragraph 1)

* * *

It is hoped that the problem of poor cellular transport will soon be resolved, thereby removing perhaps the biggest hurdle to progress in antisense chemotherapy and, especially, to the development of antisense imaging. [emphasis added] (page 696 col. 2, paragraph 3)

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The purpose of this article was to provide a brief description of antisense chemotherapy and to address the question of whetehr antisense localization can be applied to nuclear medicine imaging. Clearly, antisense imaging would be an extremely valuable diagnositc tool, since, in theory, almost any tissue or disease staet could be seletively imaged. **As this contribution may make clear, however, many improvements in the current state of antisense localization will be needed to reach this nirvana**. [emphasis added] (page 703. col 2, paragraph 2)

* * *

Existing methods of radiolabeling DNAs with gamma emitters may possible decrease cell membrane transport, interfere with mRNA binding or show intracellular instabilities leading to prolonged nonspecific retention. Alternaitve methods of radiolabeling will then be needed. . [emphasis added] (page 703. col 2, paragraph 3)

Hnatowich thus clearly teaches that antisense imaging is fraught with difficulties that have not yet been overcome. Moreover, Hnatowich provides a litany of difficulties so that, even if the combination of cited references taught or suggested the presently claimed method (which they do not as indicated above), there would still be **no reasonable expectation of success**.

Tavitan *et al.* also leads one of skill in the art to the conclusion that there is **no reasonable expectation of success** that *in vivo* imaging of gene expression in a brain cell is possible using the constructs described in the present application. Tavitan *et al.* disclose the systemic administration to a baboons of a radiolabeled nucleic acid that has no complementary sequence in mammalian cDNA databases. Moreover, Tavitan *et al.* states:

> During the first 5 min after injection (Fig. 1a-c) the radioactivity was high in the heart liver and kidney, and **low in other organs such as the brain** and muscles. [emphasis added] (page 468, col. 1, paragraph 2).
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> * * *
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> As ON1 is an "orphan" sequence with no biological target in the baboon**, the present radioactivity biodistrubitions reflect essentially the non-specific interactions** and the metabolic pathways of [18F]ON1. [emphasis added] (page 468, col. 2, paragraph 2).
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> * * *
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> In spite of their promises, **clinical applications of oligonucleotides are still to be awaited because a number of difficulties limit their use *in vivo***. Improvements to be achieved include **resistance to plama and tissular nucleases**, **better cell membrane penetration** (a necessary requisite as the target RNA is intraceullular), and reduced toxicity and side effects. [emphasis added] (page 470, col. 2, paragraph 2).
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> * * *
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> **Further investigations will tell us** if the present methodology, which will help to evaluate strategies for more effective delivery of antisense oligonucleotides to target tissues, also represents the first step toward **nucleic acid imaging** with PET. [emphasis added] (page 470, col. 2, paragraph 3).

Like Hnatowich, Tavitan *et al.* clearly teaches that effective imaging has not been accomplished and there are numerous difficulties to be overcome. Tavitan *et al.* thus clearly establishes that there is no reasonable expectation of success for the presently claimed method.

The combination of the cited references thus fails to produce teach or suggest the presently claimed method. Moreover, these references clearly establish that there is no reasonable expectation of success for the presently claimed method. Accordingly the Examiner has failed to make his *prima facie* case, and the rejection under 35 U.S.C. §103(a) should be withdrawn.

In view of the foregoing, Applicants believes all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. Should the Examiner seek to maintain the rejections, Applicants request a telephone interview with the Examiner and the Examiner's supervisor.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 769-3513.

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